

We claim:

1. A method for diagnosing disease in a test subject comprising:
  - a. obtaining a biological specimen from a test subject, said specimen comprising a mixed cell population suspected of containing intact rare cells and further comprising:
    - i. cell fragments derived from rare cells, or
    - ii. cellular debris derived from rare cells;
  - b. preparing a magnetically-labeled sample wherein said biological sample is mixed with magnetic particles coupled to a first biospecific ligand which reacts specifically with said intact rare cells, and said cell fragments or said cellular debris, to the substantial exclusion of other specimen components;
  - c. contacting said magnetically-labeled sample with at least one additional biospecific ligand which specifically labels said intact rare cells, and said cell fragments or said cellular debris, to the substantial exclusion of other specimen components;
  - d. analyzing said labeled rare cells, and said labeled cell fragments or said labeled cellular debris, the presence of said labeled rare cells, said labeled cell fragments, and said labeled cellular debris indicating the presence of disease.
2. The method of Claim 1, wherein said biological specimen is blood.
3. The method of Claim 2, wherein after said biological specimen obtained, it is contacted with an agent capable of stabilizing said biological specimen.
4. The method of Claim 1, wherein said magnetic particles are colloidal.
5. The method of Claim 1, wherein after the step of preparing said magnetically-labeled sample, said sample is subjected to a high gradient magnetic field to produce a separated magnetically-labeled fraction which is enriched for said intact rare cells, and said cell fragments or said cellular debris.
6. The method of Claim 1, wherein said analysis is selected from the group consisting of: multiparameter flow cytometry, immunofluorescent microscopy, laser scanning cytometry, bright field base image analysis, capillary volumetry, spectral imaging analysis, manual cell analysis, and automated cell analysis.

7. The method of Claim 1, wherein said analysis is based on at least one of the group consisting of: morphologic analysis and epitopic analysis.

8. A method for diagnosing disease in a test subject comprising:

- 5           a. obtaining a biological specimen from a test subject, said specimen comprising a mixed cell population suspected of containing intact rare cells and clusters of rare cells;
- b. preparing a magnetically-labeled sample wherein said biological sample is mixed with magnetic particles coupled to a first biospecific ligand which reacts  
10           specifically with said intact rare cells and said clusters of rare cells, to the substantial exclusion of other specimen components;
- c. contacting said magnetically-labeled sample with at least one additional biospecific ligand which specifically labels said intact rare cells and said clusters of rare cells, to the substantial exclusion of other specimen components;
- 15           d. analyzing said labeled rare cells and said labeled clusters of rare cells, the presence of said labeled rare cells and said labeled clusters of rare cells indicating the presence of disease.

9. The method of Claim 8, wherein said biological specimen is blood.

10. The method of Claim 9, wherein after said biological specimen obtained, it is contacted  
20           with an agent capable of stabilizing said biological specimen.

11. The method of Claim 8, wherein said magnetic particles are colloidal.

12. The method of Claim 8, wherein after the step of preparing said magnetically-labeled sample, said sample is subjected to a high gradient magnetic field to produce a separated magnetically-labeled fraction which is enriched for said intact rare cells and said clusters  
25           of rare cells.

13. The method of Claim 8, wherein said analysis is selected from the group consisting of: multiparameter flow cytometry, immunofluorescent microscopy, laser scanning cytometry, bright field base image analysis, capillary volumetry, spectral imaging analysis, manual cell analysis, and automated cell analysis.

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14. A method for diagnosing malignancy in a test subject comprising:

- a. obtaining a biological specimen from a test subject, said specimen comprising a mixed cell population suspected of containing intact malignant cells and further comprising:
- 5           i. cell fragments derived from malignant cells, or
- ii. cellular debris derived from malignant cells;
- b. preparing a magnetically-labeled sample wherein said biological sample is mixed with magnetic particles coupled to a first biospecific ligand which reacts specifically with said intact malignant cells, and said cell fragments or said cellular debris, to the substantial exclusion of other specimen components;
- 10   c. contacting said magnetically-labeled sample with at least one additional biospecific ligand which specifically labels said intact malignant cells, and said cell fragments or said cellular debris, to the substantial exclusion of other specimen components;
- 15   d. analyzing said labeled malignant cells, and said labeled cell fragments or said labeled cellular debris, the presence of said labeled malignant cells, said labeled cell fragments, and said labeled cellular debris indicating the presence of malignancy.
15. The method of Claim 14, wherein said biological specimen is blood.
16. The method of Claim 15, wherein after said biological specimen obtained, it is contacted
- 20       with an agent capable of stabilizing said biological specimen.
17. The method of Claim 14, wherein said magnetic particles are colloidal.
18. The method of Claim 14, wherein after the step of preparing said magnetically-labeled sample, said sample is subjected to a high gradient magnetic field to produce a separated magnetically-labeled fraction which is enriched for said intact malignant cells, and said
- 25       cell fragments or said cellular debris.
19. The method of Claim 14, wherein said analysis is selected from the group consisting of: multiparameter flow cytometry, immunofluorescent microscopy, laser scanning cytometry, bright field base image analysis, capillary volumetry, spectral imaging analysis, manual cell analysis, and automated cell analysis.
- 30   20. The method of Claim 14, wherein said analysis further comprises classifying cell fragments or said cellular debris based on their origin as caused by apoptosis or necrosis.

21. The method of Claim 20, wherein analysis further comprises classifying cell fragments or said cellular debris based on their origin as caused by mechanical damage, drug-induced damage, or immunological damage.

22. The method of Claim 20, wherein said classification is based on at least one of the group consisting of: morphologic analysis and epitopic analysis.

23. A method for diagnosing malignancy in a test subject comprising:

- a. obtaining a biological specimen from a test subject, said specimen comprising a mixed cell population suspected of containing intact malignant cells and clusters of malignant cells;
- b. preparing a magnetically-labeled sample wherein said biological sample is mixed with magnetic particles coupled to a first biospecific ligand which reacts specifically with said intact malignant cells and said clusters of malignant cells, to the substantial exclusion of other specimen components;
- c. contacting said magnetically-labeled sample with at least one additional biospecific ligand which specifically labels said intact malignant cells and said clusters of malignant cells, to the substantial exclusion of other specimen components;
- d. analyzing said labeled malignant cells and said labeled clusters of malignant cells, the presence of said labeled malignant cells and said labeled clusters of malignant cells indicating the presence of malignancy.

24. The method of Claim 23, wherein said biological specimen is blood.

25. The method of Claim 24, wherein after said biological specimen obtained, it is contacted with an agent capable of stabilizing said biological specimen.

26. The method of Claim 23, wherein said magnetic particles are colloidal.

27. The method of Claim 23, wherein after the step of preparing said magnetically-labeled sample, said sample is subjected to a high gradient magnetic field to produce a separated magnetically-labeled fraction which is enriched for said intact malignant cells and said clusters of malignant cells.

28. The method of Claim 23, wherein said analysis is selected from the group consisting of: multiparameter flow cytometry, immunofluorescent microscopy, laser scanning

cytometry, bright field base image analysis, capillary volumetry, spectral imaging analysis, manual cell analysis, and automated cell analysis.

29. A method for screening malignancy in a test subject comprising:

- 5           a. obtaining a biological specimen from a test subject, said specimen comprising a mixed cell population suspected of containing intact malignant cells and further comprising:
  - i. cell fragments derived from malignant cells, or
  - ii. cellular debris derived from malignant cells;
- 10          b. preparing a magnetically-labeled sample wherein said biological sample is mixed with magnetic particles coupled to a first biospecific ligand which reacts specifically with said intact malignant cells, and said cell fragments or said cellular debris, to the substantial exclusion of other specimen components;
- 15          c. contacting said magnetically-labeled sample with at least one additional biospecific ligand which specifically labels said intact malignant cells, and said cell fragments or said cellular debris, to the substantial exclusion of other specimen components;
- 20          d. analyzing said labeled malignant cells, and said labeled cell fragments or said labeled cellular debris, the presence of said labeled malignant cells, said labeled cell fragments, and said labeled cellular debris indicating the presence of malignancy.

30. The method of Claim 29, wherein said biological specimen is blood.

31. The method of Claim 30, wherein after said biological specimen obtained, it is contacted with an agent capable of stabilizing said biological specimen.

25          32. The method of Claim 29, wherein said magnetic particles are colloidal.

33. The method of Claim 29, wherein after the step of preparing said magnetically-labeled sample, said sample is subjected to a high gradient magnetic field to produce a separated magnetically-labeled fraction which is enriched for said intact malignant cells, and said cell fragments or said cellular debris.

30          34. The method of Claim 29, wherein said analysis is selected from the group consisting of: multiparameter flow cytometry, immunofluorescent microscopy, laser scanning

cytometry, bright field base image analysis, capillary volumetry, spectral imaging analysis, manual cell analysis, and automated cell analysis.

35. The method of Claim 29, wherein said analysis further comprises classifying cell fragments or said cellular debris based on their origin as caused by apoptosis or necrosis.

5 36. The method of Claim 35, wherein analysis further comprises classifying cell fragments or said cellular debris based on their origin as caused by mechanical damage, drug-induced damage, or immunological damage.

37. The method of Claim 35, wherein said classification is based on at least one of the group consisting of: morphologic analysis and epitopic analysis.

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38. A method for screening malignancy in a test subject comprising:

- a. obtaining a biological specimen from a test subject, said specimen comprising a mixed cell population suspected of containing intact malignant cells and clusters of malignant cells;
- 15 b. preparing a magnetically-labeled sample wherein said biological sample is mixed with magnetic particles coupled to a first biospecific ligand which reacts specifically with said intact malignant cells and said clusters of malignant cells, to the substantial exclusion of other specimen components;
- c. contacting said magnetically-labeled sample with at least one additional  
20 biospecific ligand which specifically labels said intact malignant cells and said clusters of malignant cells, to the substantial exclusion of other specimen components;
- d. analyzing said labeled malignant cells and said labeled clusters of malignant cells, the presence of said labeled malignant cells and said labeled clusters of malignant  
25 cells indicating the presence of malignancy.

39. The method of Claim 38, wherein said biological specimen is blood.

40. The method of Claim 39, wherein after said biological specimen obtained, it is contacted with an agent capable of stabilizing said biological specimen.

41. The method of Claim 38, wherein said magnetic particles are colloidal.

30 42. The method of Claim 38, wherein after the step of preparing said magnetically-labeled sample, said sample is subjected to a high gradient magnetic field to produce a separated

magnetically-labeled fraction which is enriched for said intact malignant cells and said clusters of malignant cells.

43. The method of Claim 38, wherein said analysis is selected from the group consisting of: multiparameter flow cytometry, immunofluorescent microscopy, laser scanning  
5 cytometry, bright field base image analysis, capillary volumetry, spectral imaging analysis, manual cell analysis, and automated cell analysis.

44. A method for monitoring malignancy in a test subject comprising:

- 10 a. obtaining a biological specimen from a test subject, said specimen comprising a mixed cell population suspected of containing intact malignant cells and further comprising:
  - i. cell fragments derived from malignant cells, or
  - ii. cellular debris derived from malignant cells;
- 15 b. preparing a magnetically-labeled sample wherein said biological sample is mixed with magnetic particles coupled to a first biospecific ligand which reacts specifically with said intact malignant cells, and said cell fragments or said cellular debris, to the substantial exclusion of other specimen components;
- 20 c. contacting said magnetically-labeled sample with at least one additional biospecific ligand which specifically labels said intact malignant cells, and said cell fragments or said cellular debris, to the substantial exclusion of other specimen components;
- 25 d. analyzing said labeled malignant cells, and said labeled cell fragments or said labeled cellular debris, the presence of said labeled malignant cells, said labeled cell fragments, and said labeled cellular debris indicating the presence of malignancy.

45. The method of Claim 44, wherein said biological specimen is blood.

46. The method of Claim 45, wherein after said biological specimen obtained, it is contacted with an agent capable of stabilizing said biological specimen.

47. The method of Claim 44, wherein said magnetic particles are colloidal.

30 48. The method of Claim 44, wherein after the step of preparing said magnetically-labeled sample, said sample is subjected to a high gradient magnetic field to produce a separated

magnetically-labeled fraction which is enriched for said intact malignant cells, and said cell fragments or said cellular debris.

49. The method of Claim 44, wherein said analysis is selected from the group consisting of: multiparameter flow cytometry, immunofluorescent microscopy, laser scanning  
5 cytometry, bright field base image analysis, capillary volumetry, spectral imaging analysis, manual cell analysis, and automated cell analysis.

50. The method of Claim 44, wherein said analysis further comprises classifying cell fragments or said cellular debris based on their origin as caused by apoptosis or necrosis.

51. The method of Claim 50, wherein analysis further comprises classifying cell fragments or  
10 said cellular debris based on their origin as caused by mechanical damage, drug-induced damage, or immunological damage.

52. The method of Claim 50, wherein said classification is based on at least one of the group consisting of: morphologic analysis and epitopic analysis.

15 53. A method for monitoring malignancy in a test subject comprising:

- a. obtaining a biological specimen from a test subject, said specimen comprising a mixed cell population suspected of containing intact malignant cells and clusters of malignant cells;
- b. preparing a magnetically-labeled sample wherein said biological sample is mixed  
20 with magnetic particles coupled to a first biospecific ligand which reacts specifically with said intact malignant cells and said clusters of malignant cells, to the substantial exclusion of other specimen components;
- c. contacting said magnetically-labeled sample with at least one additional  
25 biospecific ligand which specifically labels said intact malignant cells and said clusters of malignant cells, to the substantial exclusion of other specimen components;
- d. analyzing said labeled malignant cells and said labeled clusters of malignant cells, the presence of said labeled malignant cells and said labeled clusters of malignant cells indicating the presence of malignancy.

30 54. The method of Claim 53, wherein said biological specimen is blood.



55. The method of Claim 54, wherein after said biological specimen obtained, it is contacted with an agent capable of stabilizing said biological specimen.

56. The method of Claim 53, wherein said magnetic particles are colloidal.

57. The method of Claim 53, wherein after the step of preparing said magnetically-labeled sample, said sample is subjected to a high gradient magnetic field to produce a separated magnetically-labeled fraction which is enriched for said intact malignant cells and said clusters of malignant cells.

58. The method of Claim 53, wherein said analysis is selected from the group consisting of: multiparameter flow cytometry, immunofluorescent microscopy, laser scanning cytometry, bright field base image analysis, capillary volumetry, spectral imaging analysis, manual cell analysis, and automated cell analysis.

59. A kit for assaying a biological specimen for the presence of malignant cells, and cell fragments derived from malignant cells or cellular debris derived from malignant cells, comprising:

a. coated magnetic nanoparticles comprising:

- i. a magnetic core material,
- ii. a protein base coating material, and
- iii. an antibody that binds specifically to a first characteristic determinant of said malignant cell, and said cell fragments or said cellular debris, wherein said antibody is coupled to said base coating material;

b. at least one antibody having binding specificity for a second characteristic determinant of said malignant cell, and said cell fragments or said cellular debris;

c. an agent capable of staining further features of said malignant cells, and said cell fragments or said cellular debris.

60. The kit of Claim 59, further comprising a panel of antibodies each specific for a different characteristic determinant.

61. The kit of Claim 59, further comprising a specific agent capable of labeling non-target entities.